

# Genome Sequence of the Spinosyn-Producing Bacterium *Saccharopolyspora spinosa* NRRL 18395<sup>▽</sup>

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***Saccharopolyspora spinosa* is a Gram-positive bacterium that produces spinosad, a well-known biodegradable insecticide that is used for agricultural pest control and has an excellent environmental and mammalian toxicological profile. Here, we present the first draft genome sequence of the type strain *Saccharopolyspora spinosa* NRRL 18395, which consists of 22 scaffolds.**

*Saccharopolyspora spinosa* belongs to the genus *Saccharopolyspora*, originally isolated from a soil sample collected from the Caribbean islands in 1982 (12). *S. spinosa* is the only bacterium that secretes spinosad, a natural pesticide consisting of spinosyn A and spinosyn D that kills most pests and has no toxic effect on humans (3, 14, 15). Spinosad is produced by Dow AgroSciences, which won the Presidential Green Chemistry Challenge Award for Designing Greener Chemicals in 1999, and is classified by the U.S. Environmental Protection Agency as an environmentally and toxicologically reduced risk material.

The whole-genome sequencing of *S. spinosa* will help scientists to better understand the regulation of the spinosyn biosynthesis pathway and its relationship to other genes, which will in turn allow more sophisticated strategies to improve the production of spinosad. According to the Genomes OnLine Database (GOLD) (10), genome sequencing of this organism has not been done yet, and there is only one completed and one draft sequence available for the genus *Saccharopolyspora*, specifically, that of *Saccharopolyspora erythraea*.

Here we present the whole-genome sequence of *Saccharopolyspora spinosa* NRRL 18395 (= CGMCC 4.1365), obtained using a whole-genome shotgun strategy with an Illumina genome analyzer (totaling ~2,906.97 Mb; ~330-fold coverage of the genome) and by Roche 454 pyrosequencing (totaling ~202.28 Mb; ~23.5-fold coverage of the genome) at the Beijing Genomics Institute.

The Illumina pair-end reads were assembled by SOAPdenovo (9), while 454 reads were assembled using Newbler Assembler software. The two assembled scaffolds were combined by sequence overlapping. We also filled hundreds of gaps by PCR followed by sequencing of the PCR product with the ABI 3730 platform, producing a final draft assembly of 22 scaffolds.

The annotation was done by using MetaGeneAnnotator (13) to predict the open reading frame, by using tRNAscan-SE 1.21 (11) to find tRNA, by using RNAmmer 1.2 (8) to search rRNA, and by using Tandem Repeats Finder 4.04 (1) to find tandem repeat sequence. In addition, the contigs were searched against the KEGG (5), Pfam (2), COGs (16), and NCBI NR databases to annotate the genome.

The draft genome includes 8,581,920 bases and contains 8,302 predicted coding sequences (CDSs), with a G+C content of 67.94%. There are single-copy genes predicted for 16S and 23S rRNA, duplicated genes predicted for 5S rRNA, and 50 copies predicted for tRNAs. An estimated 82.8% of nucleotides are predicted to encode proteins. We also predicted 615 genes related to regulatory function and identified 913 tandem repeat regions. The CDSs annotated by COGs can be classified into 23 COG (clusters of orthologous groups) categories and 1,845 COGs, and 2962 CDSs can be annotated in the KEGG orthology system.

The following are present in the genome sequence: genes in the spinosyn cluster (*spnA*, *spnB*, *spnC*, *spnD*, and *spnE*), the PKS-encoding genes (17); the 4'-, 2'-, and 3'-O-methyltransferase genes (*spnH*, *spnI*, and *spnK*, respectively [7]); *spnJ*, which has catalytic function during the synthesis of the precursor of the tricyclic nucleus of spinosyns (6); and *spnQ*, which is responsible for the biosynthesis of D-forosamine (4).

**Nucleotide sequence accession number.** This whole genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number AEYC00000000.

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